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09/808,382	03/14/2001	Benjamin Eithan Reubinoff	14418	1139

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EXAMINER
TON, THAIAN N

ART UNIT	PAPER NUMBER
1632	

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10

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/808,382	REUBINOFF ET AL..	
	Examin r	Art Unit	
	Thaian N. Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 October 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-85* is/are pending in the application.

4a) Of the above claim(s) *1-7,28-38 and 69-85* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *8-27 and 39-68* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *14 March 2001* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). *3* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-85 are pending. Claims 8-27 and 39-68 are under current examination. Claims 1-7, 28-38 and 69-85 are withdrawn from consideration.

Sequence Compliance

This application fails to comply with requirement of 37 CFR 1.821-1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures. Each nucleotide and/or amino acid in the instant application must be accompanied by "SEQ ID NO:". See p. 63, lines 1, 2, and 4; p.68, Table; p. 72, lines 2-5, of the application. Applicant must comply with the requirements of the sequence rules (37 CFR 1.821-1.825).

Note that a reply to a notice to comply with the sequence rules should NOT be sent to the 20231 zip code address for the United States Patent and Trademark Office.

Please direct all replies to the United States Patent and Trademark Office via one (1) of the following:

1. Electronically submitted through EFS-Bio (<http://www.uspto.gov/ebc/efs/downloads/documents.htm>, EFS Submission User Manual - ePAVE)

2. Mailed to:
U.S. Patent and Trademark Office
Box Sequence, P.O. Box 2327
Arlington, VA 22202

3. Mailed by Federal Express, United Parcel Service or other delivery service to:
U. S. Patent and Trademark Office

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Crystal Plaza Two, Lobby, Room 1B03
Arlington, Virginia 22202

4. Hand Carried directly to the Customer Window at:
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Election/Restrictions

Applicant's election with traverse of Group II, [Claims 8-27 and 39-68] in Paper No. 9 is acknowledged. The traversal is on the ground(s) that the claimed subject matter is directed to an independent and distinct invention as defined in 35 USC §121. Applicants argue that the subject matter of Group I is drawn to enriched populations of human undifferentiated ES cells which are capable of proliferation *in vitro* and differentiation to neural progenitor cells, neuron cells or glial cells required in Group II, and the method of preparing undifferentiated human ES cells for differentiation into neural progenitor cells in Group II. Applicants further argue that the subject matter of Group II is drawn to differentiated committed human progenitor cell line(s) capable of differentiation into mature neurons or glial cells, said cell line derived from undifferentiated human ES cells from Group I. Applicants argue that accordingly, Group I and II are interrelated and interdependent and further, that the methods of Groups III-V embody the concept of Group I as these groups use the cells derived from Group I [see p. 4 of Applicants' Response]. Applicants further argue that the subject matter

in Groups II and III and between Groups II and IV are interrelated and interdependent, for example, Group II contains the source and protocol required for the ES cells in Group IV. Applicants argue that the subject matter in Groups III and either of Groups IV-V are interdependent and interrelated, for example the method of Group V embodies the concept of Groups IV and III and the method of Group IV embodies the concept of Group III [see p. 5, 1st ¶ of the Response]. Applicants further argue that the subject matter in Groups IV and V are functionally related, *i.e.,* inducing somatic cells *in vivo* from embryonic stem cells derived from human somatic precursors.

Applicants' arguments have been considered, however, they are not found persuasive because it is maintained that Groups I-V of the Restriction requirement represent distinct inventions. For example, Group I, which is drawn to enriched preparations of human undifferentiated ES cells can have separate uses than the differentiated human neural progenitor cells of Group II, as advanced on pages 2-4 of the Restriction requirement. MPEP §801.02-802.02 discusses the term *distinct* as, "two or more subjects as disclosed are related ... but are capable of separate manufacture, use or sale as claimed, AND ARE PATENTABLE (novel and unobvious) OVER EACH OTHER (though they may each be unpatentable because of the prior art). Further, it is stated that restriction includes the practice of requiring election between *distinct* inventions. In the instant case, Applicants argue that the subject matter between I and II are "interdependent and

interrelated" and that the methods of Groups III-V embody the concept of Group I [see p. 4 of the Response]. It is noted that although Groups I-V are interrelated, they are not interdependent, as the human undifferentiated ES cells of Group I are not absolutely required for the inventions of Groups II-V.

Applicants further argue that the Restriction Requirement is not in compliance with the MPEP because Groups II-V are classified in the same class subclasses and Groups I and Groups II-V are classified within the scope of the subclasses of Groups II-V. Applicants point to MPEP §808.02 to support that when the classification is the same and the field of the search is the same; no reasons exist for diving among related inventions. It is noted that the Restriction requirement is not based solely upon search burden. In fact, MPEP §803.01 states that the two criteria required for a proper Restriction requirement are that the inventions are independent or distinct as claimed and there must be a serious burden upon the examiner if restriction is required. For the reasons advanced on pages 2-4 of the Restriction requirement, the Examiner has shown that Groups I-V represent independent or distinct inventions and further, it is reiterated that the inventions above have acquired a separate status in the art as a separate subject for inventive effort and require independent searches. The search for each of the above inventions is not co-extensive particularly with regard to the literature search. Further, a reference which would anticipate the invention of one group would not necessarily anticipate or even make obvious another group.

The requirement is still deemed proper and is therefore made FINAL.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Claims 1-7, 28-38 and 69-85 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected group(s), there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 9.

Priority

Receipt is acknowledged of papers submitted under 35 U.S.C. 119(a)-(d), which papers have been placed of record in the file.

Drawings

Applicant(s) is/are hereby notified that the required timing for correction of drawings has changed. See the last 6 lines on the sheet, which is attached, entitled "Attachment for PTO-948 (Rev. 03/01 or earlier)". Due to the above notification Applicant(s) is/are required to submit drawing corrections with the time period set

for responding to this Office action. Failure to respond to this requirement may result in abandonment of the instant application or a notice of a failure to fully respond to this Office action.

Specification

The disclosure is objected to because of the following informalities:

p. 76, line 26 recites "Clumpswere".

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 8-27, 39-49, 52, 64-68 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for differentiated committed progenitor cell lines capable of propagation into mature neurons or glial cells, wherein the cell lines are derived from undifferentiated pluripotent human ES cells, enriched populations of such cell lines, methods of inducing somatic differentiation of pluripotent stem cells *in vitro* into progenitor cells, and methods for producing an enriched preparation of human ES-derived neural progenitor cells, the specification does not reasonably provide enablement for committed progenitor

cell lines of the type claimed which are derived from undifferentiated human ES cells, enriched populations and methods of using the same . The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claimed invention is directed to differentiated committed progenitor cell lines capable of differentiation and propagation into mature neurons or glial cells, the cell lines derived from undifferentiated human embryonic stem [ES] cells [claims 8-27]. In further embodiments, the claimed invention is directed to methods of inducing somatic differentiation of stem cells *in vitro* into progenitor cells [claims 39-49], and methods of preparing an enriched preparation of human ES-derived neural progenitor cells [claims 64-68].

The claims encompass both pluripotent and totipotent human ES cells. However, the specification is not enabling for the breadth of the claims. In particular, the state of the art is such that ES cell technology is generally limited to the mouse system at present, and that only "putative" ES cells exist for other species (see Moreadith *et al.*, **J. Mol. Med.**, 1997, p. 214, S*ummary*). Note that "putative" ES cells lack a demonstration of the cell to give rise to germline tissue or the whole animal, a demonstration which is an art-recognized property of ES cells. Moreadith *et al.* supports this observation as they discuss the historical perspective of mouse ES cells as follows:

"The stage was set-one could grow normal, diploid ES cells in culture for multiple passages without loss of the ability to contribute to normal development. Furthermore, the cells contributed to the development of gametes at a high frequency (germline competence) and the haploid genomes of these cells were transmitted to the next generation. Thus, the introduction of mutations in these cells offered the possibility of producing mice with a predetermined genotype."

Such a demonstration has not been provided by the specification or the prior or post-filing art with regard to the generation of <u>any</u> species of animal ES cells, other than the mouse, which can give rise to the germline tissue of a developing animal. In addition, prior to the time of filing, Mullins *et al.* (**Journal of Clinical Investigation**, 1996) report that "although to date chimeric animals have been generated from several species including the pig, in no species other than the mouse has germline transmission of an ES cell been successfully demonstrated." (page 1558, column 2, first paragraph). As the claims are drawn to methods involving the manipulation of animal embryonic stem (ES), and particularly since the subject matter of the specification and the claimed invention encompasses the use of such cells for the generation of a transgenic animal, the state of the art supports that only <u>mouse ES cells</u> were available for use for production of transgenic mice.

This is further supported by Pera *et al.* [**Journal of Cell Science** 113: 5-10 (2000)] who present the generic criteria for pluripotent ES or EG cells [see p. 6, 2nd column] and state that, "Thus far, <u>only</u> mouse EG or ES cells meet these generic

criteria. Primate ES cells meet the first three of the four criteria, but not the last. Numerous other candidate mammalian ES cells have been described over the years in domestic and laboratory species, but only in the mouse have all criteria been met rigorously." [See p. 6, 2nd column, last paragraph].

With regard to claim 14, the claim recites that the neural progenitor cell is capable of transdifferentiation into other cell lineages to generate stem cells and differentiated cells of non-neural phenotype. Although the specification broadly teach that the cells of the instant invention would be capable of transdifferentiation [see p. 21, lines 11-14, p. 51, lines 1-3], the specification does not provide specific teachings or guidance to show that the cells of the instant invention would be capable of such transdifferentiation. The state of the art of transdifferentiation is unpredictable. For example, Kennea *et al.* [**Pediatr. Res.** (2002) 52:320-1] teach that, "[T]he concept of transdifferentiation is not universally accepted. Recently, two independent studies published in the same issue of Nature have provided an alternative explanation for transdifferentiation and raised doubts as to whether it really occurs." [See p. 320, 2nd column, 1st ¶, lines 6-14]. Kennea further review the paper of Ying *et al.*, who took neural stem cells and co-cultured them with modified ES cells to investigate if the ES cells could influence the de-differentiation of committed neural stem cells to a more pluripotent phenotype. Kennea teach that following the co-culture, ES cells were selected against and then ES-like cells emerged from the neural cell cultures [see p. 320, 3rd column, 1st full ¶]. These ES-

like cells appeared to be cells that had been de-differentiated from the neural stem cells, as they expressed ES cell markers and phenotypes. However, it was noted that the resulting cells additionally were resistant to hygromycin and sensitive to ganciclovir, and that the resulting "de-differentiated" cells were possibly neural stem cell/ES cell hybrids, with different morphology than native ES cells, and were mostly tetraploid [see p. 321, 1st column, 1st ¶]. Kinnea conclude that, "Whether transdifferentiation is a true property of progenitor cells or a confusing artefact, the possibility of cell fusion must be examinined in future research claiming to demonstrate cell plasticity." [See p. 321, 2nd column]. The unpredictability in the art of neural stem cell transdifferentiation is further supported by Kennea *et al.* [**J. Pathol.** (2002) 197:536-550] who state that although somatic cells have potential to be reprogrammed to a less differentiated state, the mechanisms which underlie this process are not yet understood [see p. 542, col. 1, 1st ¶] and that the concept of stem cell transdifferentiation is not universally accepted [see p. 542, 2nd col. 2nd full ¶]. As such, with respect to the unpredictable nature of transdifferentiation of neural progenitor cells, and particularly when taken with the specification's lack of teachings or sufficient to show that the claimed neural progenitor cells could transdifferentiate into cell lineages which would generate stem cells and/or differentiated cells of a non-neural phenotype, it would <u>not</u> be predictable that the progenitor cells of the instant invention would transdifferentiate into cell lineages

which would generate stem cells and/or differentiated cells of a non-neural phenotype.

Accordingly, in view of the unpredictable and undeveloped state of the ES cell art and unavailability of any totipotent ES cells, for the breadth claimed, and lack of guidance or teachings provided by the specification for totipotent ES cells isolated from any species for the breadth claimed, the unpredictable state of the art of transdifferentiation of neural progenitor cells, and as well as the lack of guidance or teachings, or working examples provided by the specification to show that the described neural progenitor cells could transdifferentiate into other cell lineages to generate stem cells and differentiated cells of non-neural phenotypes, it would have required undue experimentation for one skilled in the art to use the claimed methods.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 8, as written, is unclear. The claim recites that the cell line is "derived from" undifferentiated human ES cells. It is unclear what the metes and bounds of "derived from" are. Claims 9 and 10 depend from claim 8.

Claims 8, 12-14, 17, 19, 22, 24-26, 40, 49 and 54 as written, are indefinite. The claims recite that the cells are capable of various characteristics [*e.g.,*

differentiation, proliferation, etc.]. It is unclear whether these characteristics actually occur or that the cells could potentially do these described things. "Capable of" implies a latent property and the conditions for the latent property must be clearly defined. Therefore, it is unclear if the latent property is ever obtained. Claim 9 depends from claim 8.

Claim 14, as written, is unclear. The claim recites that the neural progenitor cells are capable of transdifferentiation, "into other cell lineages" [see line 2 of the claim]. It is unclear what "other cell lineages" encompasses. For example, not all cell lineages would generate stem cells.

Claim 18, as written, is indefinite. The claim recites that the neural progenitor cell can incorporate extensively. It is unclear what the metes and bounds of the term "extensively" encompass. For example, what percentage or amount of a recipient brain would have to be incorporated into the recipient brain to constitute extensive incorporation? Claims 19-22 depend from claim 18.

Claim 20, as written, is unclear. The claim recites that the cell is "responsive" to host environmental signals. It is unclear what the term "responsive" encompasses. How, or in what way, does the cell respond to environmental signals? Claims 21 and 22 depend from claim 20.

Claim 23 recites the limitation "the enriched preparation" in line 1 of the claim. There is insufficient antecedent basis for this limitation in the claim. Claims 24-27 depend from claim 23.

Claim 24, as written, is indefinite. The claim recites that the cells are capable of prolonged undifferentiated proliferation. It is unclear what the term "prolonged" encompasses, what time frame does the term encompass?

Claim 27, as written, is indefinite. The claim recites that cells "may be" recovered from cryopreservation. It is unclear whether these characteristics actually occur or that the cells could potentially be recovered. "May be" implies a latent property and the conditions for the latent property must be clearly defined. Therefore, it is unclear if the latent property is ever obtained.

Claim 39, as written, is unclear. The claim recites a method of "somatic differentiation of stem cells" in line 1 of the claim. It is unclear what "somatic differentiation" encompasses. How are the cells induced to 'somatically' differentiate? The claim is further unclear. The claim recites that a "differentiating signal" is provided. It is unclear what a "differentiating signal" encompasses. Further, the claim recites the term "and/or", which is not clear if it is meant to further limit the claim. Claims 40-49 depend from claim 39.

Claim 42, as written, is indefinite. The claim recites that the cells maintain a diploid karyotype during "prolonged" cultivation. It is unclear what time frame "prolonged" encompasses.

Claim 46, as written, is indefinite. The claim recites culturing the undifferentiated stem cells for "prolonged periods" [see line 3 of the claim]. It is unclear what time frame the term "prolonged" encompasses.

Claim 50, as written, is indefinite. The claim recites that a method of "inducing somatic cells" in the preamble of the claim. It is unclear how somatic cells would be "induced", although the ES cell-derived somatic cell progenitors would be induced to differentiate into somatic cells. Claims 51-63 depend from claim 50.

Claim 58, as written, is indefinite. The claim recites "said somatic cells induced". This is unclear because the method of claim 50 refers to a method of inducing the differentiation of ES cell derived progenitor cells to somatic cells. The somatic cells are not induced.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 8-13, 15-27, 47-50, 52-54, 58 are rejected under 35 U.S.C. 102(b) as being anticipated by Flax *et al.* [**Nat. Biotech** (1998) 16:1033-1039].

The claims are directed to differentiated committed human progenitor cell lines capable of differentiation and propagation into mature neurons or glial cells, wherein the cell line is derived from undifferentiated human ES cells. In further

embodiments, the claims are directed to methods of inducing somatic cells from embryonic stem cell derived somatic progenitors.

Note that claims 11-13, 52 are product-by-process claims. The claims are directed to a neural progenitor cell differentiated *in vitro* from an undifferentiated embryonic stem cell and an embryonic stem-cell derived progenitor cell. Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. See In re Ludtke, supra. Whether the rejection is based on "inherency" under 35 USC 102, on "prima facie obviousness" under 35 USC 103, jointly or alternatively, the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products. In re Best, Bolton, and Shaw, 195 USPQ 430, 433 (CCPA 1977) citing In re Brown, 59 CCPA 1036, 459 F.2d 531, 173 USPQ 685 (1972). Further, see MPEP §2113, "Even though product-by process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process."

Further, it is noted that some of the instant claims recite that the differentiated committed human progenitor cell line is "derived from" undifferentiated human ES cells [see claim 8], or the production of somatic cells from "embryonic stem cell derived somatic progenitors" [see claim 50]. It is noted that all human cells are considered "derived" from undifferentiated human ES cells, and further, that these claims are considered product-by-process claims [see *above*].

Claims 15 and 16 recite that the differentiated neuronal progenitor cell expresses markers of neuroectodermal lineage or markers of neural progenitor cells. These markers are inherent properties of neuronal progenitor cells. It is noted that, "Products of identical chemical composition can not have mutually exclusive properties." A chemical composition and its properties are inseparable. Therefore, if the prior art teaches the identical chemical structure, the properties applicant discloses and/or claims are necessarily present. In re Spada, 911 F.2d 705, 709, 15 USPQ2d 1655, 1658 (Fed. Cir. 1990).

Flax teach stable clones of neural stem cells [NSCs] which were isolated from the human fetal telecephalon. They teach that these cells give rise to all fundamental neural lineages *in vitro*, following transplantation into germinal zones of newborn mouse brains they can participate in aspects of normal development, including migration along established migratory pathway, are cryopreservable, and can be propagated by both epigenetic and genetic means [see *Abstract*]. In particular, Flax teach that the NSC clones grew in culture as clusters that could be

passaged weekly for at least one year, and these cells expressed vimentin, a neural progenitor marker. Flax teach that the human NSCs were dissociated and plated on poly-L-lysine coated slides to induce differentiation. Further, they teach that by dissociation of the clusters, the clones differentiated spontaneously into neurons and oligodendrocytes. After 5 days these cells became immunoreactive for the neuronal marker neurofilament, and 10% expressed CNPas, a marker for oliodendrologia. [See p. 1034, 1st column *Multipotency and self-renewal in vitro* and Fig. 2].

The human NSC clones were also analyzed to see if they would respond to normal developmental cues *in vivo*, including appropriate migration, integration into host parenchyma and the differentiation into neural cell types appropriate to the region's stage of development. In particular, human NSCs were engrafted into the subventricular germinal zone [SVZ] of newborn mice and the migration and differentiation of these cells were analyzed. [See p. 1035, 2nd col.; last ¶, and Fig. 4]. It was found that human NSCs integrated into the SVZ within 48 hours of implantation and these engrafted human NSCs migrated out along the subcortical white matter by 2 weeks following engraftment. Further, it was found that the donor-derived human cells had migrated along the RMS [see Figure 4D & E and pp. 1036-1037, bridging ¶]. Three weeks following transplantation, a subpopulation of human-specific neurofilament-postive cells were present in the parenchyma of the olfactory bulb [see Fig. 5B-G] and these cells reacted with an antibody to mature

neuronal marker, NeuN. Flax teach that these engrafted human NSC clones gave rise to all three fundamental neural lineages: neurons, oligodendrocytes and astrocytes, and transplanted brains appeared normal and the animals exhibited no indications of neurological dysfunction [see p. 1037, 1st column, 1st full ¶].

Accordingly, Flax anticipate the claimed invention.

Claims 39-49 are rejected under 35 U.S.C. 102(b) as being anticipated by Thomson [US Pat No. 5,843,780, published December 1, 1998].

The claims are directed to methods of inducing somatic differentiation of stem cells *in vitro* into progenitor cells comprising obtaining undifferentiated embryonic stem cells, providing a differentiating signal under conditions which are non-permissive for stem cell renewal, do not kill cells, and/or induces unidirectional differentiation towards extraembryonic lineages.

Claims 44 and 45 recite undifferentiated ES cells prepared by the method of either claim 28 or 37, respectively. Thus, the ES cells recited in these claims are product-by-process claims [see *supra*]. Note that with regard to claims 40 and 41, which discuss the expression of various undifferentiated embryonic stem cell markers, these markers are inherent properties of undifferentiated ES cells. That is, that, "Products of identical chemical composition can not have mutually exclusive properties." A chemical composition and its properties are inseparable. Therefore, if the prior art teaches the identical chemical structure, the properties

applicant discloses and/or claims are necessarily present. In re Spada, 911 F.2d 705, 709, 15 USPQ2d 1655, 1658 (Fed. Cir. 1990).

Thomson teach the isolation of primate embryonic stem cells which are characterized by the cell surface markers: SSEA-1 (0); SSEA-3(+), SSEA-4 (+); TRA-1-60(+); TRA-1-81(+) and alkaline phosphatase (+). These cells have normal karyotypes, continue to proliferate in an undifferentiated state, and can form trophoblast and are able to differentiate into all three embryonic gem layers [see *Abstract*]. Thomson teach methods of isolating primate embryonic stem cells, comprising isolating a primate blastocyst, isolating cells from the inner cell mass [ICM] of the blastocyst, plating the ICM cells on a fibroblast layer, wherein ICM-derived cell masses are formed, removing an ICM-derived cell mass and dissociating the mass into dissociated cells, replating the dissociated cells on embryonic feeder cells, and selecting colonies with compact morphology containing cells with high nucleus/cytoplasm ratio, and prominent nucleoli. [See col. 4, lines 33-44]. Thomson teach that by manipulation of culture conditions, the primate ES cells can be induced to differentiate into specific cell types, such as neuron cells [see col. 6, lines 9-12]. Thomson teach that the when grown on embryonic fibroblasts and allowed to grow for two weeks after achieving confluence, primate ES cells will spontaneously differentiate [see col. 12, lines 53-59]. They further teach that SCID mice were injected with the primate embryonic stem cells intra-testicularly and it was found that tumors were formed and demonstrated differentiation into derivatives of all

three embryonic germ layers, including ganglia, glia, neural precursors, etc. [see col. 15, lines 30-40].

Accordingly, Thomson anticipate the claimed invention.

Claims 8-13, 15-27, 50-54 and 58 are rejected under 35 U.S.C. 102(b) as being anticipated by Vescovi *et al.* [**Exp. Neurol** (March 1999) 156:71-83].

Vescovi teach human neural embryonic stem cells which were isolated from post-conception human diencephalon. Single cells from mechanically dissociated tissue were cultured in the absence of growth factors, or in the presence of EGF or FGF2 [either alone, or in combination]. These cultures were serially passaged and differentiation was initiated by transferring single stem cells into polyorthinine-coated tissue culture wells in growth medium. The stem cells were seen to proliferate and give rise to spherical clones, and these were further sub-cloned to generate cultures that were differentiated in control medium [see p. 72, col. 1, last ¶ and col. 2]. Vescovi teach that the single cells were plated in NS-A basal serum-free medium, and for differentiation studies, were plated on polyornithine-coated coverslips in DMEM/F12 serum-free medium [see p. 80, 2nd column, 1st ¶]. The human CNS stem cells were differentiated for 6 days and the resulting neurons and glia were implanted in rat brains. These animals were analyzed and it was found that the injected human cells had survived and some had migrated on average of 1.2 mm rostrocaudally and 0.75 mm mediolaterally from the grafting site [see p. 77,

1st column ¶ 1-2]. Vescovi teach that the human CNS stem cells were cryopreserved [see p. 80, last ¶] and then thawed [see p. 81, 1st ¶].

Accordingly, Vescovi anticipate the claimed invention.

Claims 8, 10-13, 15, 16, 23-25, 50, 52-55, 58 and 59 are rejected under 35 U.S.C. 102(b) as being anticipated by Anderson *et al.* [**U.S. Pat. No. 5,693,482**, published December 2, 1997].

Note that with regard to claims 8, 10-13, which recite that the human prognitor cell lines are 'derived from' undifferentiated human ES cells, all human cells are 'derived from' undifferentiated human ES cells. With regard to claims 15 and 16, which recite neural progenitor cell markers, these are inherent properties of neural progenitor cells, see *supra*.

Anderson teach mammalian multipotent neural stem cells, methods of isolation and differentiation of such cells [see *Abstract*]. In particular, they teach that the neural stem cells are cultured in feeder cell-independent cultures [see col. 2, lines 46-49] and are allowed to differentiate into progenitor cells, as well as differentiated neurons and glia of the peripheral nervous system [see col. 2, lines 55-62]. Anderson teach that these neural crest stem cells can be cultured on a mixed substrate of poly-D-lysine and fibronection to induce differentiation [see col. 3, lines 48-51]. They teach that neural stem cells can be cultured on any

substratum for the attachment of the cells, including fibronectin [col. 8, lines 21-38, col. 14, lines 1-12].

Accordingly, Anderson anticipate the claimed invention.

Claims 8, 11-13, 15, 16, 23-25, 50-54 and 58 are rejected under 35 U.S.C. 102(a) as being anticipated by Johansson *et al.* [**Exp. Cell Res.** (Dec. 1999) 253:733-736].

The claims are directed to a differentiated committed human progenitor cell line capable of differentiation and propagation into mature neurons or glial cells, wherein the cell line is derived from undifferentiated human ES cells [claims 8, 11-13, 15, and 16] and enriched preparations of neural progenitor cells [claims 23-25]. In further embodiments, the claims are directed to methods of inducing somatic cells from ES cell derived somatic progenitors comprising obtaining a source of ES cell derived somatic progenitors, culturing the progenitor cells on an adhesive substrate, and inducing the cells to differentiate to somatic cells under conditions which favor somatic differentiation, and in further embodiments, the ES cells derived somatic cell progenitor cells are grown in the presence of a serum free median and growth factors, and are induced to differentiate by withdrawal of the growth factors [claims 50-54].

Note that with regard to claim 8, the recitation of "derived from undifferentiated human ES cells," all human cells are derived from undifferentiated

human ES cells. Furthermore, with regard to claims 15 and 16, the markers of neural progenitor cells are inherent properties of the cells [see *supra*]. Furthermore, with regard to claim 50, the term "embryonic stem cell derived" in line 1 of the claim, it is noted, as above, all human cells are derived from ES cells.

Johansson teach that hippocampal and lateral ventricle wall tissue was obtained and cell cultures were made. In particular, the cells were cultured in DMEM/F12 medium, wherein bFGF and EGF had been added [see p. 734, 1st col. 2nd ¶]. They teach that differentiation of neurospheres was induced by transferring the neurospheres onto poly-L-ornithine coated glass slides in the previously described medium without growth factors [see p. 734, 1st col. 4th ¶]. It was found that when the neurospheres were induced to differentiate, all contained neurons, astrocytes and oligodendrocytes [see Figure 2 and p. 734, 2nd column].

Accordingly, Johansson teach the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 64-68 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson [**US Pat No. 5,843,780**, published December 1, 1998] when taken with Johansson *et al.* [**Exp. Cell Res.** (Dec. 1999) 253:733-736].

The claims are directed to methods of producing an enriched preparation of human ES derived neural progenitor cells said method comprising recovering undifferentiated human ES cells from the inner cell mass cells from a human embryo, inducing differentiation of the ES cells to neural progenitor cells and identifying neural progenitor cells by expression of neural-specific markers, and culturing the neural progenitor cells to promote proliferation and propagation. In further embodiments, the neural progenitor cells are cultured as spheres or monolayers in serum-free medium comprising DMEM/F12 supplemented with growth factors including B27, EGF and bFGF.

Thomson teach the isolation of primate embryonic stem cells which are characterized by the cell surface markers: SSEA-1 (0); SSEA-3(+), SSEA-4 (+); TRA-1-60(+); TRA-1-81(+) and alkaline phosphatase (+). These cells have normal karyotypes, continue to proliferate in an undifferentiated state, and can form trophoblast and are able to differentiate into all three embryonic gem layers [see *Abstract*]. Thomson teach methods of isolating primate embryonic stem cells, comprising isolating a primate blastocyst, isolating cells from the inner cell mass [ICM] of the blastocyst, plating the ICM cells on a fibroblast layer, wherein ICM-derived cell masses are formed, removing an ICM-derived cell mass and dissociating the mass into dissociated cells, replating the dissociated cells on embryonic feeder cells, and selecting colonies with compact morphology containing cells with high nucleus/cytoplasm ratio, and prominent nucleoli. [See col. 4, lines 33-44]. Thomson teach that by manipulation of culture conditions, the primate ES cells can be induced to differentiate into specific cell types, such as neuron cells [see col. 6, lines 9-12]. Thomson teach that the when grown on embryonic fibroblasts and allowed to grow for two weeks after achieving confluence, primate ES cells will spontaneously differentiate [see col. 12, lines 53-59]. They further teach that SCID mice were injected with the primate embryonic stem cells intra-testicularly and it was found that tumors were formed and demonstrated differentiation into derivatives of all three embryonic germ layers, including ganglia, glia, neural precursors, etc. [see col. 15, lines 30-40].

Thomson differs from the claimed invention in that they do not teach culturing neural progenitor cells as spheres or monolayers in serum-free medium comprising DMEM/F12 supplemented with growth factors which include B27, EGF and bFGF. However, prior to the time the claimed invention was made, Johansson the culturing of human neural stem cells in DMEM/F12 medium, wherein bFGF, B27 supplement and EGF had been added [see p. 734, 1st col. 2nd ¶]. They further teach that neurospheres were passaged, they were dissociated into single cells and the single cells were picked with a micropipette and transferred to microwells [see p. 734, 3rd ¶].

Accordingly, in view of the combined teachings of Thomson and Johansson, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to culture the neural progenitor cells as taught by Thomson in the culturing conditions as taught by Johansson, with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to make such a modification because it was an art-recognized goal to optimize the culturing of stem cells, and in particular, as taught by Johansson that the culture conditions employed provide methods to maintain and propagate neural stem cells [see p. 733, col. 1-2, bridging ¶].

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claim 56 is rejected under 35 U.S.C. 103(a) as being unpatentable over Anderson [**U.S. Pat. No. 5,693,482**, published December 2, 1997].

The claim is directed to methods of inducing somatic cells from ES cell derived somatic progenitor cells, wherein the progenitor cells are cultured on poly-D-lysine and laminin.

Anderson teach mammalian multipotent neural stem cells, methods of isolation and differentiation of such cells [see *Abstract*]. In particular, they teach that the neural stem cells are cultured in feeder cell-independent cultures [see col. 2, lines 46-49] and are allowed to differentiate into progenitor cells, as well as differentiated neurons and glia of the peripheral nervous system [see col. 2, lines 55-62]. Anderson teach that these neural crest stem cells can be cultured on a mixed substrate of poly-D-lysine and fibronection to induce differentiation [see col. 3, lines 48-51].

Anderson differ from the claimed invention in that they do not specifically teach the culturing of the neural stem cells on laminin. However, they teach that neural stem cells can be cultured on any substratum for the attachment of the cells, [col. 8, lines 21-38, col. 14, lines 1-12], and it is well-known in the art to culture cells on adhesive substrates such as fibronectin, or laminin.

Accordingly, in view of Anderson, it would have been obvious for one of ordinary skill in the art at the time the claimed invention was made, to modify the

culturing steps using fibronectin, as taught by Anderson to substitute laminin, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as it was well-known in the art to culture cells on adhesive substrates such as laminin. Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claim 57 is rejected under 35 U.S.C. 103(a) as being unpatentable over Anderson [U.S. Pat. No. 5,693,482, published December 2, 1997] when taken with van Inzen *et al.* [Biochimica et Biophys Acta (1996) 1312:21-26].

The claim is directed to methods of inducing somatic cells from ES cell derived somatic progenitor cells, wherein the progenitor cells are cultured on poly-D-lysine and laminin and further culturing the cells in the presence of retinoic acid.

Anderson is described *supra*. They differ from the claimed invention in that they do not teach further culture of the progenitor cells in the presence of retinoic acid. However, prior to the time the claimed invention was made, van Inzen teach that neuronal differentiation of precursor cells can be stimulated by the treatment of the cells with retinoic acid [see *Abstract* and p. 21, 2nd column, 1st ¶]. In particular, they teach that by culturing ES cells in retinoic acid treatment up to

50% of the population stained positive for the neuronal specific GAP-43 antibody [see p. 24, 1st col., 1st ¶].

Accordingly, in view of the combined teachings of Anderson and van Inzen, it would have been obvious for one skilled in the art to modify the culturing techniques to induce differentiation of neural crest stem cells as taught by Anderson by additionally culturing the stem cells in retinoic acid, as taught by van Inzen, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as the culturing of stem cells in retinoic acid, as taught by van Inzen, would improve the differentiation of the stem cells to mature neurons and that using retinoic acid is a simple way to induce neuronal differentiation [see p. 26, 1st column, last ¶].

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Thaian N. Ton whose telephone number is (703) 305-1019. The examiner can normally be reached on Monday through Friday from 8:00 to 5:00 (Eastern Standard Time), with alternating Fridays off. Should the examiner be unavailable, inquiries should be directed to Deborah Reynolds, Supervisory Primary Examiner of Art Unit 1632, at (703) 305-4051. Any administrative or procedural questions should be directed to Tiffiany Tabb, Patent Analyst, at (703) 605-1238. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 872-9306.

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